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(54) Title: INJECTABLE TISSUE RECONSTRUCTION MATERIAL

(57) Abstract

An injectable and flowable material for tissue repair, reconstruction and bulking is provided, comprising microspheres having an average diameter in the range of about 10 to 100 microns. The close packing of the microspheres at the site of application and the biodegradation of the materials allow for residency of the particles at the target site to permit tissue repair and in–growth into the implant. Active components, such as drugs or growth factors may be incorporated into the material to elicit a specific tissue response or to provide local delivery of a therapeutic agent.

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INJECTABLE TISSUE RECONSTRUCTION MATERIAL

This is a continuation-in-part of Serial No. 08/923,623, filed September 4, 1997, which is incorporated by reference in its entirety.

The invention is directed to compositions which are biodegradable and injectable for implantation into tissues to facilitate repair, reconstruction and bulking of damaged or deficient tissue areas in the body.

Background of the Invention

There are several situations which require the efficient repair, reconstruction and augmentation of biological tissues. For example, during surgery, various materials such as sutures, staples, tissue adhesives, natural and synthetic polymers and autologous tissues have been used to provide reconstructive scaffolds for the repair of damaged tissues or to bulk tissues into the appropriate anatomically correct configuration. In these applications the use of degradable materials allows the reconstruction to be performed without a follow-up surgical procedure to remove non-degraded materials which may provoke long term tissue irritation or infection. However, when utilizing the techniques in the field of minimally invasive surgery, methods to augment and repair tissues through small bore cannulae are required in order to permit surgical repair with lower operational time and cost.

There are certain diseases and conditions which may be treated or remedied by tissue reconstruction. In one example, tissue segments of the myocardium (heart muscle) are damaged and possibly rendered completely non-viable as a result of myocardial infarction. Utilizing a degradable reconstruction material tailored to provide a

scaffold for tissue repair as well a reservoir for substances to stimulate various healing responses, these damaged areas may be re-vitalized to provide some utility in maintaining coronary output. There are no materials other than difficult to obtain tissue grafts to repair non-viable cardiac tissue.

Another example pertains to urinary incontinence due to intrinsic sphincter deficiency (ISD) where the urethral sphincter is partially or totally incompetent, causing leakage of urine from the bladder. There are several modalities used to treat ISD, including external devices, such as pads and diapers, and more complex internal devices, including surgical intervention to correct the anatomic defects. Sphincter augmentation involves the injection of a substance into the tissues surrounding the urethra in order to increase the periurethral tissue mass, bringing the tissues into apposition and therefore increasing resistance to urine flow. Such substances which have been tested for this procedure include autologous fat, silicon elastomer particles, Teflon particles and dispersions of bovine derived collagen. The biodegradable materials which have been used, such as collagen, require re-injection periodically because of their shorter residence time as compared to non-biodegradable materials, such as silicon and Teflon.

Other utilizations for tissue reconstruction materials include the repair of depressed scars or wrinkles on the skin, repair of bone fractures or defects, repair of damaged cartilage tissues and the embolization of vascular deformities.

Summary of the Invention

An injectable and flowable material for tissue reconstruction is provided comprising microparticles,

preferably microspheres formed of a biomaterial which sets at the site of application into a cohesive mass containing an interconnected porous network. The biomaterial is biodegradable, can be delivered at a high solids content and is cross-linked to a sufficient extent such that the *in vivo* residency of the particles at the site of application is sufficient to stimulate tissue repair and in-growth. The extent of cross-linking may be adjusted to tailor the material for specific applications. The microparticles may optionally contain active compounds or drugs to stimulate tissue repair such as the incorporation of growth or angiogenic factors, or the incorporation of anti-infective or anti-inflammatory agents to limit adverse tissue response.

A method of inducing tissue repair in a tissue defect or wound site is provided comprising the step of applying an effective amount of the described injectable compositions to a site of desired tissue reconstruction .

In particular, a method is provided to induce tissue repair and in-growth to treat damaged myocardial tissue, structurally defective sphincters and vocal cords, depressed scars or skin wrinkles, damaged bone or cartilage and vascular deformities.

Detailed Description of the Invention

The invention provides an injectable composition and methods of its use for repairing damaged or incompetent tissues by administering via injection an effective amount of biomaterial at the site of the defect or structural deformity.

The terms "damaged" and "incompetent" are used to describe tissues damaged due to vascular ischemia; muscles, tendons, or cartilage that are inadequately functioning or non-functioning due to deterioration; or a

structural deformity caused by an anatomic disruption of a muscle or mechanism.

The term "effective amount" means the quantity of biomaterial needed to repair tissue or to achieve improved continence, or the quantity of wound healing agents needed to achieve improved healing. The effective amount of biomaterial administered may vary depending upon the patient's ability to absorb or breakdown the biomaterial, the consistency and concentration of the material, and the site and condition being treated. The biomaterial may be administered over a number of treatment sessions to achieve and maintain the desired results.

The biomaterial used in the present invention is in a form which is injectable, biocompatible, non-immunogenic and in a physical and chemical state that allows it to persist at the site of placement for at least 7 days. These include, but are not limited to, biodegradable materials such as collagen, gelatin, elastin, fibrin, fibrinogen, glycosoaminoglycans, polyhydroxybutyrate, polylactic acid, polyglycolic acid, polyesters and combinations of these materials.

Highly purified, high molecular weight gelatin or collagen is particularly useful. Commercially available laboratory grades may be used, or gelatin derived from collagen obtained by known purification processes of collagenous materials available from sources such as bovine or porcine corium, bone or tendon. It is preferred that the gelatin material be of high molecular weight of at least 200 to 300 bloom and substantially free of extraneous proteins, proteoglycans, lipids or other processing residuals which may adversely effect biological response.

The biomaterial will be in the form of solid or hollow microparticles which can be delivered to the

target site as a high solids content slurry without the inherent high viscosity found in dispersions and emulsions of other particulates. Typically, the microparticles will be in the form of microspheres. The microspheres are stabilized by cross-linking, so that they are insoluble in an aqueous media. Typically, the diameters of the microspheres will be in the range of about 10 to 100 microns, and can be conveniently injected through a cannula having an inner diameter of 10x the average microsphere diameter.

The microspheres may be produced by spray drying, spray coagulation or emulsion methods. Microspheres produced by spray drying are usually hollow whereas those produced by spray coagulation are usually solid. While the usual particle diameter will be in the range of about 10 to 100 microns, particles in the range of 10 to 55 microns are also useful, particularly for treating tissues through small bore needles.

For a gelatin raw material, the biomaterial will typically be solubilized in an aqueous solution, either water or a buffered aqueous solution, ranging from about pH 2 to pH 10 depending on the source of the gelatin and isoelectric point. The gelatin concentration in the solution is typically between about 2 and 20 percent weight/volume.

The microspheres are cross-linked to increase their stability and resistance to *in-vivo* degradation. Various cross-linking procedures may be utilized, either singly or in combination, including but not limited to, cross-linking using carbodiimides, aldehydes, dehydrothermal (DHT) or other methods known to those skilled in the art. The extent of cross-linking can be adjusted to achieve the desired *in-vivo* degradation rate of the material, with higher cross-linked materials lasting for a longer time period than lower cross-linked materials.

A preferred method for cross-linking to achieve good tissue compatibility and medium term resorption rates is to cross-link with carbodiimides. A co-solvent system using 2-propanol (IPA) and dilute hydrochloric acid (HCL), in a concentration ranging from 1 to 100 mM, in a solution ratio ranging from 99:1% to 70:30% (IPA/HCL) is used. The cross-linking agent is then added, preferably 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), in a concentration of typically 0.5 to 5 mg/ml. The cross-linking reaction may be run for approximately 4 to 96 hours. Subsequent to cross-linking the microspheres are washed with dilute HCL, in a concentration range from 10 - 100 mM, and IPA. The microspheres are then collected and dried.

Another method for cross-linking to achieve high enzymatic resistance and low fluid uptake (swell) involves the use of two different cross-linking chemistries in succession. The first cross-linking is performed in a co-solvent system using IPA and deionized water (DI) in a solution ratio ranging from 99:1% to 70:30% (IPA/DI). The deionized water is acidified, typically with HCL, to an unbuffered pH in a range of about 4.0 to 6.5. The cross-linking agent is then added, preferably EDC, in a concentration of typically 0.5 to 5 mg/ml. Cross-linking may be run for approximately 4 to 48 hours.

The second cross-linking step is preferably accomplished in a co-solvent system using (IPA) and dilute phosphate solution (PHOS) at a concentration ranging from 0.05 to 0.5 M, at a pH ranging from 5.0 to 8.0, in a solution ratio ranging from 99:1% to 5:95% (IPA/PHOS). Glutaraldehyde (GTA) is added as the cross-linking agent in the concentration ranging from about 0.001 to 1.0 percent. This reaction is run for a period of time from about 4 to 48 hours.

Subsequent to cross-linking the microspheres are washed with de-ionized water and isopropyl alcohol, then collected and dried.

Residuals of cross-linking agents and break-down products from such agents must be sufficiently minimal so as not to adversely affect tissue response. Sterilization may be accomplished using standard practice such as electron beam irradiation, gamma irradiation or ethylene oxide gas exposure.

The cross-linked microspheres are typically formulated into a flowable slurry with a biologically acceptable vehicle, such as saline or phosphate buffer. The concentration (solids-content) range may be determined so that the flow rate is suitable for the application, which is typically in the range of about 2 to 70% weight/volume of solids. Typically, the solids content will be at least 10%, which may be delivered through cannula as small as about 30 gauge.

Additives may be incorporated into the fluid vehicle to promote flow properties of the slurry, such as, but not limited to, uncrosslinked gelatin, collagen, hyaluronic acid, polyethylene glycol, surfactants or other flow promoting agents. The fluid vehicle may also be formulated with physiologically acceptable ionic compounds to adjust for toxicologically acceptable ionic strength and pH.

While not intending to be bound by any theory, upon injection of the microsphere slurry into tissues, the microspheres are believed to aggregate into a mass having an interconnected porosity created by the close-packing of the essentially spherical particles, providing areas for cellular in-growth and the resulting tissue integration via cellular proliferation between the particles. Alternatively, some of the particles may be cross-linked to a lesser extent than the others and

therefore be susceptible to a faster degradation to allow for the creation of a network of passages within the implant to further encourage new tissue in-growth and tissue formation.

Other variations of preparation of the microspheres may be accomplished. For example, the cross-linked microspheres may be resuspended in gelatin and resprayed or recoagulated to create a larger set of particles containing a cross-linked core and an uncross-linked or lightly cross-linked outer shell. This multi-layered approach may be used for products such as tissue adhesives, where a soft and sticky outer shell is provided which allows cohesion of the particles to each other and adhesion to the tissues to which they have been applied. The multi-layered particle may also incorporate either as the core or the shell, other materials, such as polyhydroxybutyrate, polylactic acid, polyglycolic acid, polyesters, elastins, fibrin, fibrinogen or collagen.

The particles are prepared by dissolving the biomaterial raw material into solution, suitably buffered, if required, depending upon the nature of the biomaterial. In the case of gelatin, typically the solution will be 100% water or a solution buffered within the range of about pH 2 to 10. A useful concentration for forming microspheres by spraying methods is a solution having a concentration of the biopolymer in the range of about 2 - 20% weight/volume. This solution may be mixed with active ingredients such as growth factors or angiogenesis factors.

Fine sprayed droplets of the solution are thereafter formed to produce solid microspheres having average diameters in the range of about 10 to 100 microns. Suitable techniques for forming droplets include spray drying, spray coagulation, emulsification, extrusion,

electrostatic droplet formation and other known droplet forming methodology.

Typically the dispersion droplets may be sprayed through a micro-droplet forming apparatus into a non-solvent of the biomaterial that is immiscible with water, such as isopropanol, hexene or chloroform, at a temperature above the freezing point of the non-solvent in order to physically stabilize the droplets. Alternatively, the dispersion droplets may be sprayed into liquefied gas, such as liquid nitrogen, to stabilize the droplets.

Once the microspheres have been formed, cross-linking is effected to increase stability and resistance to degradation when implanted into a body. The microspheres must be of high purity materials, free of potentially toxic additives which may impair tissue growth or preclude complete resorption upon implantation. Additives such as protease inhibitors may be formulated into the microspheres either prior to or after microsphere formation to extend the residence time in-vivo.

After cross-linking the microspheres are washed to remove unbound cross-linking agent, collected and dried. Washing is conducted typically in mild acidic solutions, deionized water and/or isopropanol.

Finally, the microspheres may be sterilized using standard practices such as electron beam irradiation, gamma irradiation or ethylene oxide gas exposure. Alternatively, the raw material components may have been sterilized and the microspheres fabricated aseptically with sterile equipment.

The cross-linked microspheres are formulated into a fully flowable slurry with a biologically acceptable vehicle such as saline or phosphate buffer.

The injectable formulations may contain macromolecular materials for promoting tissue repair, ingrowth or angiogenesis. Materials may be chosen from, but not limited to, hyaluronic acid, FGF (fibroblast growth factor), TGF-beta or PDGF (platelet derived growth factor), angiogenic growth factors, vascular endothelial growth factor. Drug components loaded into the microspheres provide for a sustained delivery and biological effect, enhancing drug performance and tissue reconstruction. The formulations may also contain therapeutic agents for local delivery around the area of application. Drug components such as growth factors, protease inhibitors, anti-infective agents, anti-inflammatory agents, anti-proliferative agents, anti-tumor agents and the like may be incorporated in the materials used to fabricate the microspheres or preferably incorporated into the microspheres after fabrication and stabilization by crosslinking. The drug components may be placed in a solvent system to allow diffusion into the microspheres, utilizing ionic strength and pH conditions to control drug loading. Alternatively, the drug components may be incorporated into the fluid vehicle and allowed to diffuse into the microspheres prior to use.

The materials according to the invention are particularly applicable for repairing damaged cardiac tissue, augmenting defective urinary sphincters or vocal cords; for filling scars, skin wrinkles or puncture wounds, delivering therapeutic compounds to tumors, delivering compounds to vascular tissues to prevent restenosis and repairing damaged cartilage or ossiferous tissue. In one example, the material may be formulated with a suitable growth factor chosen for angiogenic potential and then injected into a region of non-functional myocardial tissue to promote repair of the

site after an ischemic episode. In another example, the material without any additives may be injected into the periurethral tissues under endoscopic visualization to repair incompetent urethral sphincter functionality. Multiple injections may be performed in order to bring the urethral tissues into apposition. For the repair of depressed scars or wrinkles, the microsphere formulation is injected utilizing known methods for treating cutaneous depressions as with such materials as a collagen-based commercial product. The material will provide similar tissue bulking properties.

Example 1

A solution of high purity gelatin of 300 bloom was made up at 10% weight/volume solids. The dry gelatin was added to 100% de-ionized water and placed in an oven at 60° C. for 1 hour, mixing occasionally. A spraying apparatus consisting of two co-axial stainless steel tubes was set-up. The inner tube was connected to a temperature controlled reservoir containing the gelatin solution and means to dispense the solution at a constant rate. The outer tube was connected to a pressurized gas tank, preferably nitrogen, to supply the carrier gas for spraying. The carrier gas was dispensed through a heating element attached to a temperature controller to provide constant temperature gas. The spray head was positioned above a tank containing 100% isopropyl alcohol (IPA).

The gas supply was activated and set to deliver a constant flow rate at a constant temperature and pressure. The pumping means was activated to deliver the temperature controlled solution into the spray head. The spray head produced small droplets of the gelatin solution, which coalesced into spherical droplets during their fall to the IPA bath. Upon contact with the IPA,

the water component of the droplets was removed via solvent exchange and the droplets became stabile microspheres. The microspheres were collected by passing through precision sieves to isolate the size fraction(s) desired for the product. The collected microspheres were then placed in a low humidity drying chamber until all of the IPA was evaporated.

A cross-linking solution was prepared using IPA and 1 mM HCL in a ratio of 92/8% (IPA/HCL). EDC was added at a concentration of 50 mg per ml of cross-link solution. The solution was mixed until the EDC was fully dissolved. The solution was added to a container with the dry microspheres in a concentration of 50 ml solution per gram of microspheres. The container was mixed by rotating for a period of 48 hours.

Subsequent to the cross-linking step, the solution containing cross-linked microspheres was filtered through an 8 micron filter to collect the microspheres. The microspheres were then washed to remove residual cross-linking agent. The microspheres were placed into a solution of 50 mM HCL and mixed for a period of 24 hours. The microspheres were filter collected and then placed into a solution of 100% IPA for a period of 4 hours. These washing steps were repeated one more time and then the collected microspheres were dried in a low humidity chamber.

The microspheres were packaged by weight into glass or plastic vials and sterilized using 2.5 MRad equivalent electron beam irradiation.

Example 2

Microspheres were fabricated using a spray system into an IPA bath as detailed in Example 1 above. The microspheres were cross-linked using a combination of two cross-link chemistries in succession. The first cross-linking step was accomplished according to Example 1 above. After the microspheres were collected, they were dried in a low humidity chamber in preparation for the second cross-linking step.

A solution of IPA and 50 mM phosphate solution (sodium phosphate monobasic plus sodium phosphate dibasic) at a pH of 7.0, in a solution ratio of 92/8% (IPA/Phos) was made up. Glutaraldehyde was added at a concentration of 0.1%. The microspheres were placed into a container and the cross-link solution was added in a concentration of 100 ml solution per gram of microspheres. The container was mixed by rotating for a period of 48 hours. Subsequent to the second cross-linking step, the microspheres were washed and sterilized according to Example 1 above.

Example 3

A fibrinogen based microsphere tissue adhesive was fabricated as follows. A solution of fibrinogen (Fractional type 1-S from bovine plasma, Sigma Chemicals) was made up in 100% DI at a concentration of 10% weight/volume. The fibrinogen was solubilized at a temperature of 37° C. for 25-35 minutes. The resulting solution was then spray coagulated as detailed in Example 1 above. The collected fibrinogen microspheres were then dried in a low humidity chamber until all the IPA was evaporated.

A solution was made up of bovine thrombin (Sigma Chemicals) and 40 mM calcium chloride ($CaCl_2$) at pH 7.0. 1,000 units of thrombin were added to 2 ml of the $CaCl_2$

solution. The thrombin solution was added to a sample of the fibrinogen microspheres. The fibrinogen/thrombin reaction created a firm solid mass from the slurry within 1 minute.Microscopic examination indicated a fully fused "clot" mass resulting from the mixture.

Example 4

Sterile microspheres according to Example 1 above were dispersed in phosphate buffered saline (PBS) at a solids content of 15% weight/volume. The dispersion was loaded into 1cc sterile syringes (Becton-Dickinson Corp.(BD)). 26 gage intra-dermal needles (BD) were attached to the syringes. New Zealand White rabbits, 3-4 months old, weighing 2-4 kilograms were prepared by anesthetizing with Ketamine and Xylazine, then shaving their dorsal spine from the plane of the shoulder blades distally for approximately 8 cm. The microsphere dispersions were injected in 6 locations, 3 each on either side of the spine, approximately 1-2 cm laterally from the spine. Injection of control materials comprising two commercially available tissue bulking agents (Contigen, CR Bard Inc and Fibrel, Mentor Corp.) were made in 2 locations totaling 8 injection sites. Injections of 0.25 cc of material were made at each site. The materials were injected subcutaneously in each location. A total of 8 animals were studied for time points of 7, 14, 28 and 56 days. The animals are euthanized according to protocol and the implant sites excised. At necropsy all microsphere implant sites showed remarkable coherence of implant material, with no indications of migration of the particles. Implant sites were intact at 7 and 14 days, with some sites showing resorption beginning between 14 and 28 days. By 56 days all of the sites showed some degree of degradation including complete resorption in some cases. The sites

were carefully excised and the explants placed in buffered formalin for fixation.

After a minimum of 14 days fixation, the explants were sectioned and stained for histological examination. Examination of the implant sites from 7 days implantation indicated the beginnings of cellular infiltration into the microsphere matrix. Varying degrees of inflammatory response as indicated by inflammatory cell types could be seen in each site. By the 14 day implantation, the sites showed remarkable cellular ingrowth into the microsphere matrix. Cellular infiltration between the microspheres was seen throughout the margins of the implants and appeared to be continuing at a rapid rate. By the 28 day implantation, the beginnings of microvasculature could be noted by the presence of voids in the matrix filled with red blood cells. These indications continued to the 56 day implant.

Example 5

Sterile microspheres fabricated according to Example 2 above were dispersed into syringes according to Example 4 above. The dispersion concentration was made up at 20% weight/volume solids. New Zealand White rabbits, 3-4 months old, weighing 2-4 kilograms were prepared by anesthetizing with Ketamine and Xylazine, then shaving their abdominal region from the central abdomen to the genitalia. An incision approximately 2 cm long was made beginning about 0.5 cm from the genitalia and proceeding caudally. The incision was made through the cutaneous layers. The skin was retracted and the bladder was exposed and pulled from the abdominal cavity.

Injections of the microsphere dispersions were made into the wall of the bladder using the 26 gage intra-dermal needles (BD). Injections were made into 2 sites in the bladder wall in 4 animals for time points of 7 and

14 days. Injections of 0.1 cc were made at each site. The surgical sites were closed with 2 layers continuous suture plus a cutaneous layer of interrupted sutures.

The animals were euthanized according to protocol at the proper time points. The bladders of each animal were explanted and fixed in buffered formalin for histological evaluation. The fixed bladders were sectioned and stained. Microscopic examination showed similar tissue response as in Example 4 above, however with the smaller implant quantity, indications were that full resorption of the implant would occur in fewer days than in Example 4.

Example 6

Microspheres fabricated according to Example 1 were cross-linked using different time end points to vary the extent of cross-linking. The extent of cross-linking was measured by the amount of fluid uptake (swell) experienced by the particles when hydrated with water. Samples of the fabricated microspheres were cross-linked with EDC for 24, 47 and 73 hours. The microspheres were washed and dried as in Example 1. The samples for each time point were then hydrated with an excess of water, the excess water was removed and the hydrated microspheres weighed. The samples were then dried in an oven at 120° C for 1 hour and then re-weighed to obtain the dry weights. The swell was calculated as a percentage. The microspheres cross-linked for 24 hours had a swell of 553%, those cross-linked for 47 hours had a swell of 462% and the 73 hour samples had a swell value of 441%. The extent of cross-linking of the microspheres is expected to relate to the *in-vivo* degradation rate of the material, with higher cross-linked materials lasting for a longer time period than lower cross-linked materials.

Example 7

Dispersions of microspheres fabricated according to Example 1 above were made using PBS in concentrations of 15 and 20% weight/volume solids. The dispersions were placed into 1 cc syringes and 26 gage needles attached. Commercially available dispersions of collagen (Contigen) at 3.5% weight/volume solids were placed in similar syringe/needle combinations. The force required to expel the dispersions was estimated manually. It was found that the microsphere dispersions were able to be expelled with similar forces to the commercial product even with the solids content being significantly higher.

Example 8

Sterile microspheres according to Example 1 were dispersed in phosphate buffered saline with 0.1% bovine serum albumin with varying amounts of recombinant basic fibroblast growth factor (bFGF). A slurry was prepared, composed of 17% by weight of microspheres with 0, 1, 25, or 50 micrograms of bFGF per cc. Injections of 0.2 cc of the microsphere slurry were injected through a 18 1/2 gauge needle into the subcutaneous tissue of rabbit dorsum. On one side of the spine, injections with 0, 1, 25, 50 micrograms per cc bFGF was injected, formulated in the microsphere slurry aseptically. On the other side of the spine, the corresponding doses of bFGF were injected, formulated in buffer alone. The rabbits were necropsied and the tissues around the injections examined after 7 and 14 days. The tissues exposed to bFGF in buffer alone were unremarkable and appeared normal. The microsphere samples had in all cases formed a single mass without trace particles. The samples all showed only traces of inflammation and appeared well accepted by surrounding tissue. At the 7 day examination, the microsphere samples were all approximately the same size. The

samples with bFGF appeared more translucent and attached to surrounding tissues, especially at the two highest dosages. At the 14 day examination, the microsphere samples were progressively smaller with increasing bFGF dosage as compared to the sample without bFGF. The samples had integrated with surrounding tissue, especially when samples were found partially intramuscular.

What is claimed is:

1. An injectable and flowable composition for tissue reconstruction comprising microparticles formed of a biomaterial which is biodegradable and cross-linked to a density such that the *in vivo* retention period of said particles at the site of application is sufficient to stimulate tissue repair and in-growth.

2. A composition according to claim 1 wherein said the average diameter of said microparticles is in the range of 1 to 100 microns.

3. A composition according to claim 1 wherein said microparticles are in the form of microspheres.

4. A composition according to claim 2 wherein said diameter is relatively uniform such that close packing rules apply to the packing of said microparticles at the site of application to form an integral mass with interconnecting porosity.

5. A composition according to claim 1 further comprising a biologically acceptable medium in which said microparticles are dispersed at a solids content of at least 10% weight per volume.

6. A composition according to claim 1 wherein said biomaterial is selected from the group consisting of gelatin, collagen, fibrinogen, fibrin, polylactic acid, polyglycolic acid, polyhydroxybutyrate, polyesters, elastin, glycosomaminoglycans and combinations of two or more of said biomaterials.

7. A composition according to claim 1 wherein said biomaterial comprises gelatin.

8. A composition according to claim 1 wherein said biomaterial is cross-linked with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide.

9. A composition according to claim 1 wherein said biomaterial is cross-linked with glutaraldehyde.

10. A composition according to claim 1 wherein said biomaterial is cross-linked with a combination of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and glutaraldehyde.

11. A composition according to claim 1 further comprising growth factors, protease inhibitors, anti-infective agents, anti-tumor agents, anti-proliferative agents, or anti-inflammatory agents.

12. A composition according to claim 11 comprising basic fibroblast growth factor or vascular endothelial growth factor.

13. A composition according to claim 1 in which the microparticles aggregate in-vivo to form a mass with interconnecting porosity capable of harboring tissue ingrowth.

14. A composition according to claim 1 wherein said microparticles comprise a plurality of layers.

15. A method of inducing tissue growth at a tissue defect or wound site comprising the step of applying an effective amount of an injectable composition comprising

microparticles of biomaterial which is biodegradable and cross-linked to a density such that the *in vivo* retention period of said microparticles at the site of application is sufficient to stimulate tissue repair in-growth.

16. A method according to claim 15 wherein said site is cardiac tissue.

17. A method according to claim 15 wherein said site is cartilaginous tissue.

18. A method according to claim 15 wherein said site is ossiferous tissue.

19. A method according to claim 15 wherein said site is a structurally defective sphincter.

20. A method according to claim 15 wherein said site is a vocal cord.

21. A method according to claim 15 wherein said site is a scar.

22. A method according to claim 15 wherein said site is a skin wrinkle.

23. A method according to claim 15 wherein said site is a puncture wound.

24. A method according to claim 15 wherein said biomaterial is selected from the group consisting of gelatin, collagen, fibrinogen, fibrin, polylactic acid, polyglycolic acid, polyhydroxybutyrate, polyesters, elastin, glycosomaminoglycans and combinations of two or more of said biomaterials.

25. A method according to claim 15 wherein the average diameter of said microparticles are in the range of 1 to 100 microns.

26. A method according to claim 15 wherein said microparticles are in the form of microspheres.

27. A method according to claim 25 wherein said diameter is relatively uniform such that close packing rules apply to the packing of said microparticles at the site of application to form an integral mass with interconnecting porosity.

28. A method according to claim 15 wherein said composition further comprises a biologically acceptable medium in which said microparticles are dispersed at a solids content of at least 10% weight per volume.

29. A method according to claim 15 wherein said biomaterial comprises gelatin.

30. A method according to claim 15 wherein said biomaterial is cross-linked with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide.

31. A method according to claim 15 wherein said biomaterial is cross-linked with glutaraldehyde.

32. A method according to claim 15 wherein said biomaterial is cross-linked with a combination of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and glutaraldehyde.

33. A method according to claim 15 wherein said composition further comprises growth factors, protease

inhibitors, anti-infective agents or anti-inflammatory agents.

34. A method according to claim 15 wherein said microparticles comprise a plurality of layers.

35. A process for fabricating microspheres comprising the steps of dispersing a biomaterial into an aqueous solvent to form a dispersion, forming said dispersion into microdroplets, capturing said microdroplets in a non-solvent of said biomaterial to form said microspheres.

36. A process according to claim 35 wherein said aqueous solvent is miscible with said non-solvent.

37. A process according to claim 35 further comprising the step of cross-linking said microspheres.

38. A process according to claim 37 wherein said cross-linking is performed by contacting said microspheres with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

39. A process according to claim 37 wherein said cross-linking is performed by contacting said microspheres with glutaraldehyde.

40. A process according to claim 37 where said cross-linking is accomplish by dehydrothermal dehydration.

41. A process according to claim 37 wherein said cross-linking is performed by contacting said microspheres with a plurality of crosslinking agents.

42. A process according to claim 41 wherein said agents comprise 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and glutaraldehyde.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/18877

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61F 2/02; A61K 38/39
US CL :424/436; 514/801, 882; 530/356
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/436; 514/801, 882; 530/356

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN ON LINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 5,490,984 A (FREED) 13 February 1996, col.4, lines 18, 55-61,col. 5, line 51, paragraph bridging columns 5-6, col. 7, lines 31+.	1,6,11-13 ---------- 1,2-5,8-10
X,P ----- Y,P	US 5,752,974 A (RHEE et al) 19 May 1998, see entire document	1,6,7,11-13 ---------- 1,2-5, 8-10
X,P ----- Y,P	US 5,759,582 A (LEONG et al.) 02 June 1998, see entire document	1-4,6,7,9,11-13 ---------- 1,5,8,10

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 30 NOVEMBER 1998	Date of mailing of the international search report 17 DEC 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MICHAEL BORIN Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/18877

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,608,251 A (MIA) 26 August 1986, col. 3, lines 67-68.	1, 8-10
Y	US 5,484,735 A (DAVIS et al) 16 January 1996.	1, 8-10
A	US 5,298,243 A (IKADA et al.) 29 March 1994, see entire document.	1-13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/18877

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-14, drawn to composition.
Group II, claims 15-33, drawn to method of use.
Group III, claims 34-41, drawn to method of making.

This application contains claims directed to more than one species of the biomaterial of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid.
The species of biomaterial are as follows:
1. gelatin
2. collagen
3. fibrinogen
4. fibrin
5. polylactic acid
6. polyglycolic acid
7. polyhydroxybutyrate
8. polyesters
9. elastin
10. glycosaminoglycans

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The composition of Group I is the technical feature linking Groups I-III. The composition of Group I is not the contribution over the prior art because it is suggested by Freed et al. or is obvious over Freed et al alone or taken in combination with Mia or Davis. Therefore, the lack of unity is present because the linking technical feature is not a "special technical feature" as defined by PCT Rule 13.2. The species of Group II will be examined together with the product of Group I in the scope commensurate with the elected product species (see below). Methods of making of Group III will be examined, in the scope commensurate with the elected product species, if the appropriate additional examination fees are paid.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: This application contains inventions of Groups I-III directed to more than one species of the generic invention. These species are deemed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1. Under PCT Rule 13.2, the species lack the same or corresponding special technical features because they do not share any common structure, i.e., there is no structurally distinctive portion of the structure which is shared by all of the alternatives. A reference teaching, e.g., a composition comprising gelatin would not teach or suggest a composition comprising polyesters, and so on.

In order for more than one species to be examined, the appropriate additional examination fees must be paid.